*J. Physiol.* (1983), **334**, *pp.* 19–31
*With 5 text-figures*
*Printed in Great Britain*



# THE ANTAGONISM OF AMINO ACID-INDUCED EXCITATIONS OF RAT HIPPOCAMPAL CA1 NEURONES *IN VITRO*

BY G. L. COLLINGRIDGE, S. J. KEHL AND H. McLENNAN
*From the Department of Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5*

(*Received 1 April 1982*)

SUMMARY

1. The effects of the ionophoretic application of a number of excitatory amino acids and antagonists to the dendrites of CA1 neurones of rat hippocampal slices maintained *in vitro* were examined. Cells were excited by *N*-methyl-DL-aspartate (NMA), kainate, quisqualate, L-aspartate and L-glutamate; NMA was unique in causing cells to fire in bursts of repetitive discharges in contrast to the sustained firing seen with the other compounds.

2. D-(−)-α-aminoadipate (DAA) and (±)-2-amino-5-phosphonovalerate (APV) were selective NMA antagonists, the latter appearing to be the more potent; in addition both compounds potentiated the responses to kainate and quisqualate. L-glutamate excitations were affected less by APV than were those of L-aspartate. The antagonist properties of APV appeared to reside with the D-(−)-isomer.

3. γ-D-glutamylglycine (DGG) in low ionophoretic doses inhibited NMA-, kainate- and aspartate-induced cell firing but at higher doses the quisqualate and glutamate responses were also decreased.

4. Kainate and NMA responses were blocked by D-(−)-2-amino-4-phosphonobutyrate (D-APB) which also had some action against the excitatory effects of L-aspartate. L-APB had no antagonistic effects, but often produced potentiation of amino acid excitations or was itself an excitant.

5. The effects of NMA and those of kainate and quisqualate were blocked by (±)-*cis*-2,3-piperidine dicarboxylate (PDA), but this compound itself had a direct excitatory effect. L-glutamate diethylester (GDEE) did not show specific antagonism of any amino acid excitations.

6. DGG and APV did not affect ACh excitations and these selective antagonists should be of value in studying the involvement of the excitatory amino acids in synaptic transmission in the hippocampus. Because they are less potent and/or have complicating direct effects PDA, GDEE, D- and L-APB may be less useful in this regard.

## INTRODUCTION

On the basis of studies in the spinal cord Watkins (1981*a*, *b*) and McLennan (1981) have proposed the existence of three pharmacologically distinguishable excitatory amino acid receptors. The first type is identified by its most potent and selective

agonist, *N*-methyl-D-aspartate (NMA) and is antagonized by compounds which include the monoamino dicarboxylates D-(−)-α-aminosuberate and D-(−)-α-aminoadipate (DAA) (McLennan & Lodge, 1979; Davies & Watkins, 1979); certain ω-phosphonate compounds such as (±)-2-amino-5-phosphonovalerate (APV) (Davies, Francis, Jones & Watkins, 1981*b*), the D-(−)-isomer of which is one of the most potent NMA receptor antagonists known (Davies & Watkins, 1982); and the partial NMA agonist (±)-*cis*-2,3-piperidine dicarboxylate (PDA) (Davies, Evans, Francis, Jones & Watkins, 1981*a*; McLennan & Liu, 1982). The second receptor, preferentially activated by kainic acid, is sensitive to antagonism by γ-D-glutamylglycine (DGG) (Davies & Watkins, 1981) and PDA. A third receptor preferentially affected by L-glutamate itself and by quisqualic acid and α-amino-5-methyl-3-hydroxy-4-isoxazole-propionic acid (AMPA) (Honoré, Krogsgaard-Larsen, Hansen & Lauridsen, 1981) is insensitive to the NMA and kainate antagonists. Quisqualate (McLennan & Lodge, 1979) and AMPA (Honoré *et al.* 1981) responses in the spinal cord can be blocked by L-glutamate diethylester (GDEE).

Many of the amino acid excitants, including L-aspartate in particular, and possibly L-glutamate, are blocked by more than one of the available antagonists, indicating that these compounds behave as mixed agonists. Therefore, while differential antagonism of excitation induced either synaptically or by application of glutamate and aspartate is of interest, to be more meaningful a profile of interaction of the antagonists with each of the NMA, kainate and quisqualate receptors must be established. This pharmacological information may then be used to determine the contribution of each receptor type to excitatory synaptic responses.

In this study a pharmacological analysis of the excitatory amino acid receptors on the dendrites of CA1 hippocampal neurones was done preparatory to an examination of the synaptic effects of amino acid agonists and antagonists in this region (Collingridge, Kehl & McLennan, 1983). The hippocampal slice maintained *in vitro* was chosen because of the wealth of information concerning its neurochemistry (Nadler, Vaca, White, Lynch & Cotman, 1976; Storm-Mathisen, 1977, 1981), anatomy (Laurberg, 1979) and electrophysiology (Schwartzkroin, 1975) as well as its amenability to investigations of this sort (Dingledine, Dodd & Kelly, 1980; Lynch & Schubert, 1980). The results indicate that there are only minor differences between antagonist effects in the spinal cord and hippocampus, and that DGG and APV should be particularly useful as synaptic antagonists in the hippocampus.

Some of these results have been reported in a preliminary form (Collingridge, Kehl & McLennan, 1982).

## METHODS

Experiments were performed *in vitro* on transverse hippocampal slices obtained from male rats. After decapitation, the brain was removed and the hippocampus was dissected out. Using a McIlwain tissue chopper, slices 400 μm thick were cut and transferred to a recording chamber (Spencer, Gribkoff, Cotman & Lynch, 1976) where they were perfused (1–1·5 ml./min) with warmed (32–34 °C) Ringer solution (124 mM-NaCl, 1·25 mM-$NaH_2PO_4$, 26 mM-$NaHCO_3$, 5 mM-KCl, 2 mM-$CaCl_2$, 2 mM-$MgSO_4$, 10 mM-D-glucose maintained at pH 7·4–7·5 with $O_2/CO_2$ (95 % : 5 %)). The fluid level was adjusted to reach the surface of the slice and the chamber was continuously supplied with a warmed, humidified $O_2/CO_2$ mixture. Stable electrophysiological responses were obtainable after equilibration for one hour.

For extracellular recording of the activity of single CA1 hippocampal neurones, a 4 M-NaCl-filled

glass micro-electrode (2–5 MΩ) was placed under visual control in stratum pyramidale. Action potentials, filtered at 0·1–3 kHz, were gated with an adjustable DC level and integrated to obtain a chart record of firing frequency. Agonists and antagonists were applied ionophoretically from a seven-barrelled micro-electrode onto the apical dendrites in the stratum radiatum, 100–200 μm from the cell body layer. Excitants were applied in a time-controlled sequence with ejecting currents adjusted to produce as nearly as possible equal but submaximal firing rates; antagonists were applied in most cases to produce 50–100 % reduction of the effect of the most sensitive agonist. The following solutions were used: L-glutamate (500 mM, pH 8·0), L-aspartate (500 mM, pH 8·0), kainate (5–10 mM. pH 8·0 in 160 mM-NaCl), quisqualate (5 mM, pH. 8·0 in 160 mM-NaCl), *N*-methyl-DL-aspartate (NMA) (20–50 mM, pH 8·0 in 160 mM-NaCl), acetylcholine chloride (ACh) (500 mM, pH 4·0), (±)-2-amino-5-phosphonovalerate (APV) (20 mM, pH 8·0 in 160 mM-NaCl), L-(+)-2-amino-5-phosphonovalerate (L-APV) (20 mM, pH 8 in 160 mM-NaCl, $[\alpha]_D$ in 6 N-HCl + 24·2°), γ-D-glutamylglycine (DGG) (50-100 mM, pH 7·0 in 100 mM-NaCl, $[\alpha]_D$ in 6 N-HCl −25·2°), (±)-*cis*-2,3-piperidine dicarboxylate (PDA) (100 mM, pH 8·0 in 100 mM-NaCl), D-(−)-2-amino-4-phosphonobutyrate (D-APB) (20 mM, pH 7·5–8·0 in 160 mM-NaCl, $[\alpha]_D$ in 6 N-HCl −23·5°), L-(+)-2-amino-4-phosphonobutyrate (L-APB) (20 mM, pH 7·5–8·0 in 160 mM-NaCl, $[\alpha]_D$ in 6 N-HCl +20·8°), L-glutamate diethylester (GDEE) (500 mM, pH 3·5–4·0), D-(−)-α-aminoadipate (DAA) (200 mM, pH 8·0).

## RESULTS

The unidentified CA1 neurones examined by extracellular recording in this study were sensitive to dendritic application of the excitatory amino acids NMA, kainate, quisqualate, L-aspartate and L-glutamate. Especially with the latter three agonists it was possible to locate a dendritic site where ionophoretic ejection currents of 0–50 nA elicited the firing of action potentials, and where vertical movement of the electrode tip 25–50 μm away from the dendrite abolished or drastically reduced the neuronal response. This implies that the effects obtained were local and not due to diffusion of the ejected materials to the soma 100–200 μm away.

Although a large number of cells were examined only 129 fulfilled the criteria for selection which were: (1) that the action potential of the cell could be suitably isolated by adjustment of the DC gate; (2) that the spike height did not change appreciably; and (3) that in pharmacological tests recoveries at least to 80 % of control firing rates were attained.

These criteria were easily achieved for excitations elicited by aspartate, glutamate or quisqualate, all of which showed a short (< 2 sec) latency to onset of firing and where the rate rapidly attained a stable level which declined equally rapidly with the termination of the ejecting current. On average there was no obvious difference in the apparent potency of L-glutamate or L-aspartate as excitants when applied to the apical dendrites in the stratum radiatum. With few exceptions equal currents ejecting the two amino acids produced nearly equivalent responses. The action of kainate on the other hand had a longer latency of onset (2–10 sec). Only occasionally could a submaximal plateau of firing be attained, and more often there was a continuous slow rise in the neuronal firing rate during kainate ionophoresis.

The situation was even more difficult in the case of NMA. The usual response to this substance, which also characteristically had a long latency of onset (2–10 sec) was initially to cause the cell to fire in bursts which were succeeded by continuous firing at a high frequency (> 35 Hz) during which the amplitude of the spikes rapidly declined in height. The tendency of NMA to recruit adjacent neurones required target cells to be well isolated and carefully gated. In addition, particularly because of the

rapid spike inactivation, it was often difficult to obtain an equilibrium response with NMA. In most instances the NMA-ejecting current was stopped as the response pattern shifted from bursts to the rapidly inactivating high frequency spike train.

The specific agonists NMA, kainate and quisqualate all were clearly more potent than either L-glutamate or L-aspartate; however, since transport numbers were not determined and with the problem of achieving stable firing rates a precise rank order could not be assigned. On the basis of the ejecting currents used and the concentrations in the electrode barrels these three agonists appeared to be 20–100 times more active than the naturally occurring compounds.

TABLE 1. Effects of amino acid antagonists on the excitatory responses to NMA, kainate, quisqualate, L-aspartate, L-glutamate and ACh

| | Agonist (mean ± S.E.M of % change firing rate) | | |
|---|---|---|---|
| Antagonist | NMA | Kainate | Quisqualate |
| APV | −93·5 ± 2·2 ($n$ = 13) | +38·3 ± 8·8 ($n$ = 10) | +28·0 ± 9·0 ($n$ = 11) |
| DGG | −89·5 ± 2·6 ($n$ = 23) | −49·5 ± 5·5 ($n$ = 28) | −6·2 ± 4·2 ($n$ = 30)* |
| D-APB | −68·0 ± 3·6 ($n$ = 20) | −43·0 ± 8·0 ($n$ = 15) | −7·5 ± 5·5 ($n$ = 19)* |
| DAA | −58·9 ± 7·2 ($n$ = 16) | +38·5 ± 17·6 ($n$ = 7)* | +59·4 ± 8·7 ($n$ = 16) |
| PDA† | −52·7 ± 8·1 ($n$ = 7) | −9·9 ± 11·7 ($n$ = 7)* | +14·7 ± 14·4 ($n$ = 7)* |
| GDEE | −11·3 ± 14·7 ($n$ = 8)* | +9·5 ± 2·9 ($n$ = 9) | +14·0 ± 5·3 ($n$ = 15) |
| L-APB | +53·9 ± 15·3 ($n$ = 9) | +14·3 ± 9·5 ($n$ = 4)* | +66·9 ± 31·8 ($n$ = 9) |
| | L-aspartate | L-glutamate | ACh |
| APV | −53·3 ± 5·3 ($n$ = 10) | −12·5 ± 2·6 ($n$ = 10) | +1·4 ± 1·8 ($n$ = 5)* |
| DGG | −61·8 ± 11·2 ($n$ = 7) | −14·2 ± 10·7 ($n$ = 9)* | +2·9 ± 4·0 ($n$ = 3)* |
| D-APB | −29·8 ± 7·0 ($n$ = 8) | +3·1 ± 7·3 ($n$ = 8)* | +2·8 ± 3·7 ($n$ = 4)* |
| L-APB | +17·4 ± 13·4 ($n$ = 5)* | +30·4 ± 10·4 ($n$ = 5)* | n.t. |

* Not significantly different ($P < 0·05$) from zero.
† Applied with ionophoretic currents < 20 nA (see text).
n.t., not tested.

Cells could also be excited with ACh. The response to ACh applied with high currents (80–100 nA) had a latency 5–10 times longer than that observed with nearly equipotent doses of aspartate. In addition cell firing persisted for 20–80 sec after the ACh ejecting current was stopped (Dodd, Dingledine & Kelly, 1981).

The effects of PDA, APV, DAA, DGG, D-APB, L-APB and GDEE on the excitation of hippocampal CA1 neurones induced by NMA, kainate, quisqualate, L-aspartate, L-glutamate and ACh are summarized in Table 1. When applied with low ejecting currents (up to 20 nA) for short periods of time PDA exhibited preferential antagonism of the NMA response (Fig. 1*A*). Higher doses and/or longer periods of administration of PDA elicited potent depression of responses induced by all three agonists (Davies *et al.* 1981*a*). This antagonism was, unfortunately, complicated by an accompanying excitatory effect (Fig. 1*B*) which even at lower doses was manifested by an enhanced response to quisqualate (Fig. 1*A*). That the excitatory effect of PDA could be blocked to a large extent with APV (data not shown) is consistent with its activity as a partial NMA agonist (Davies *et al.* 1981*a*). The effects of PDA or other antagonists could not be mimicked by a 100 nA current carried by chloride ions (Fig. 1*A*).

Of the other amino acid antagonists tested only DAA and APV selectively antagonized NMA-induced excitations; of the two APV was the more potent as has been reported by others (Davies, *et al.* 1981*b*). The preferential effect of APV on the NMA response is illustrated in Fig. 2. Potentiation of the kainate- and quisqualate-induced responses was an effect noted with both APV (Fig. 2) and DAA (Table 1).

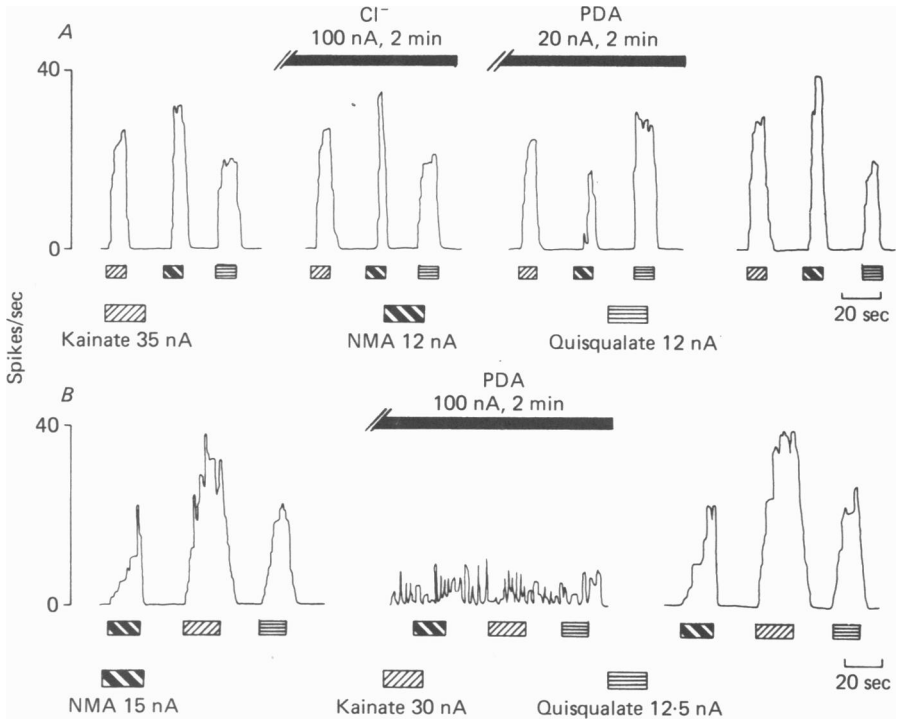


**Fig. 1.** Ratemeter records showing the effects of PDA on hippocampal CA1 neuronal responses to NMA, kainate and quisqualate. In *A* responses to NMA were reduced following a two minute application of PDA (20 nA). In record *B*, obtained from a different CA1 neurone, PDA (100 nA) antagonized responses to each of the three agonists and was itself excitatory. Excitations were not affected by a 100 nA current carried by chloride. In this and following records the upper horizontal bar identifies the antagonist, its ejecting current and the time elapsed since the start of antagonist ejection. The lower horizontal bars indicate the duration of agonist administration and the legend below identifies the agonists and their ejecting currents.

Experiments in which the effects of the DL mixture and the resolved (98%) L-(+)-isomer of APV were compared on the same cells showed that only the DL mixture was effective, implying that both the antagonist properties of APV and its potentiating effect upon quisqualate reside in the D-(−)-isomer (cf. Davies, *et al.* 1981*b*; McLennan, 1982). Thus ionophoretic ejection of DL-APV decreased the NMA response by 94·3±3·9% and increased the quisqualate response by 21·8±4·9% (mean±S.E. of the mean, $n = 4$) while L-APV ejected with current intensities equal to half of those used to apply DL-APV produced no significant effect on NMA- or quisqualate-induced responses (Fig. 3*A*). That antagonism of the NMA response

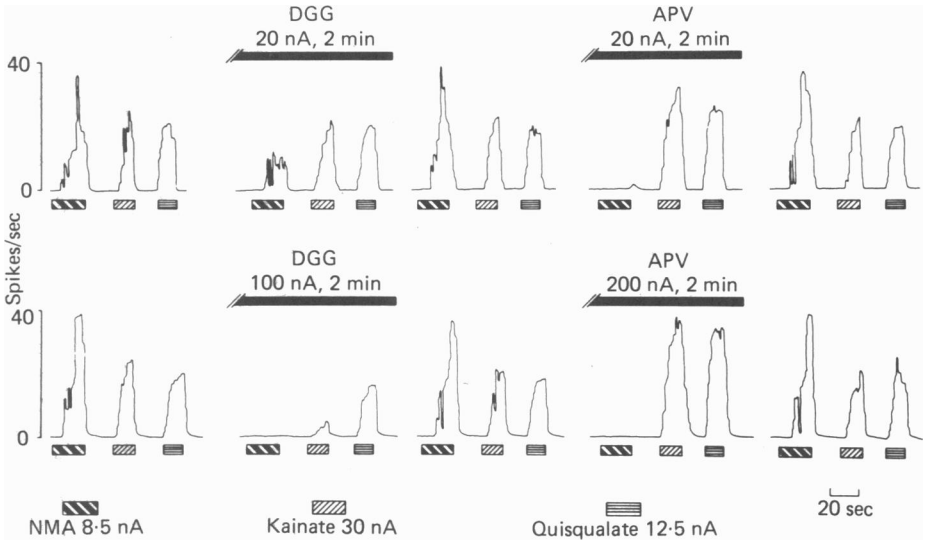


**Fig. 2. Blockade of amino acid-induced excitations by APV and DGG. APV reduced responses to NMA and potentiated quisqualate- and kainate-induced excitations while DGG antagonized NMA, kainate and quisqualate responses in that order of sensitivity.**

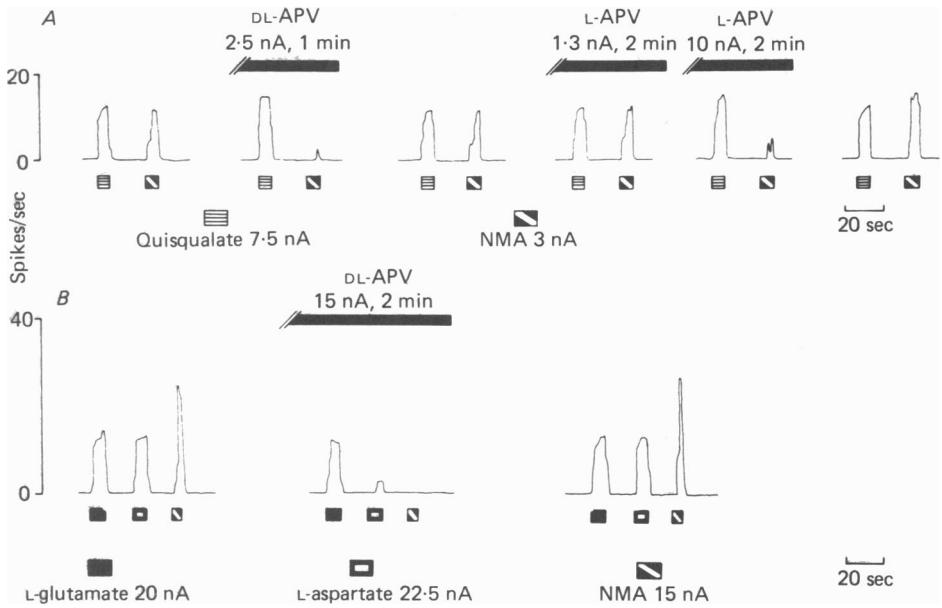


**Fig. 3. Effects of DL-APV and L-APV on responses of hippocampal CA1 neurones to excitatory amino acids. In *A* the racaemic mixture of APV decreased and increased the responses to NMA and quisqualate, respectively, effects which were reproduced only with much higher doses of L-APB. In *B* DL-APV selectively blocked excitations induced by NMA and L-aspartate.**

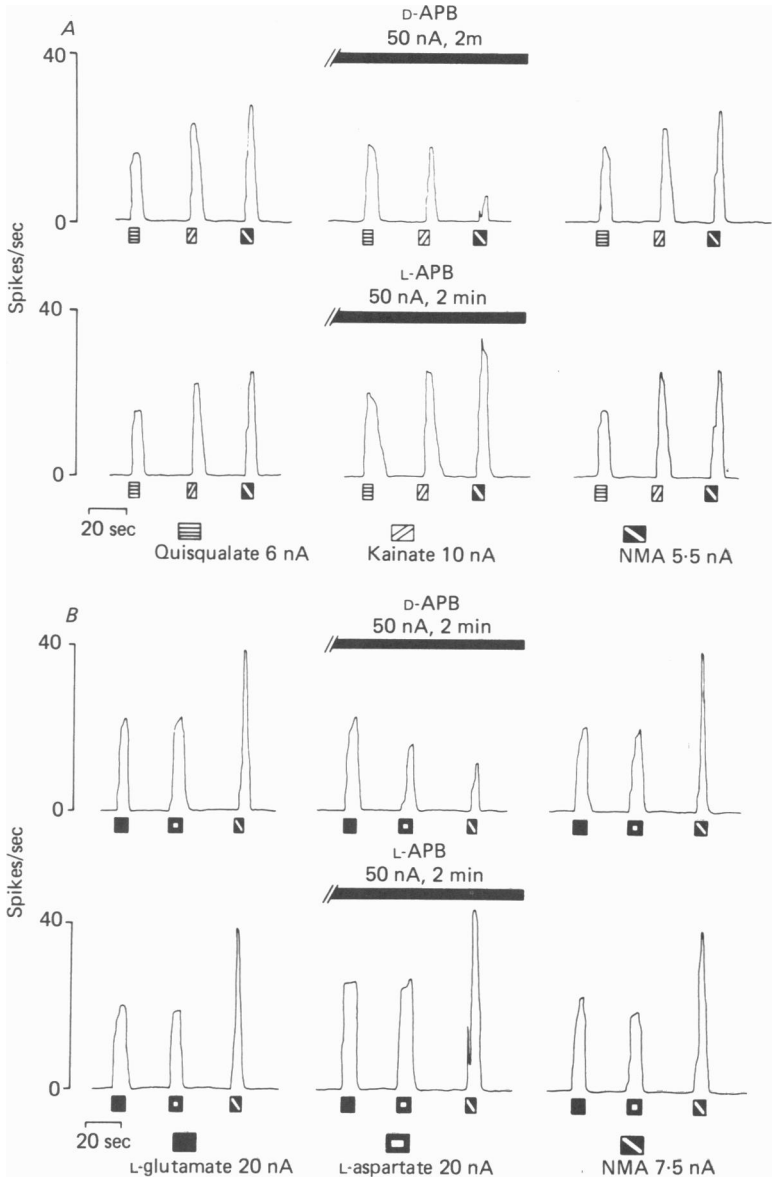


**Fig. 4. Effects of the optical isomers of APB on responses of two CA1 neurones (*A*, *B*) to ionophoresis of excitatory amino acids. NMA, L-aspartate and kainate responses were antagonized by D-APB. With both cells an increase from the control firing rate was observed in the presence of L-APB.**

could be observed with high (40–100 nA) ejection currents of L-APV (Fig. 3*A*) may be attributed to the small contamination of the L-APV sample by the active isomer.

The dipeptide DGG produced a marked depression of the NMA-induced response and also antagonized the effect of kainate, albeit to a lesser extent. Of the three specific agonists examined the responses of hippocampal CA1 neurones to quisqualate

was least affected by DGG although a high dose did have some effect (Fig. 2). When applied with high ejecting currents (75–100 nA) rather than the 15–50 nA used to obtain the data of Table 1, DGG decreased the kainate and quisqualate responses by 80·7 ± 4·9 % ($n = 6$) and 28·3 ± 8·6 % ($n = 6$) respectively.

The two isomers of APB which were resolved to approximately 90 % purity had strikingly different effects (Fig. 4*A*). D-APB antagonized the response to NMA and

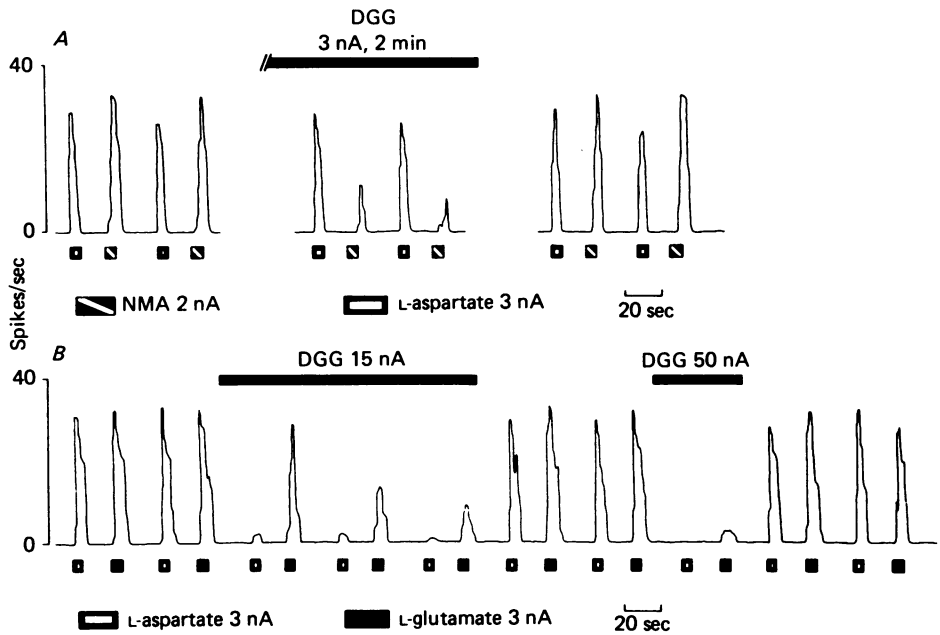


Fig. 5. The differential antagonism by DGG of the responses of CA1 neurones to NMA, L-aspartate and L-glutamate. Record *A* illustrates blockade of the NMA but not the L-aspartate response by DGG. In *B* higher doses of DGG which reduced or abolished the L-aspartate response were less effective against L-glutamate-induced excitations.

kainate while the quisqualate response remained relatively unaffected. In contrast, the L-(+)-isomer of APB potentiated the action of each of the three agonists and on occasion was itself excitatory. This excitatory effect of L-APB was sensitive to blockade by D-APB (data not shown), as was reported in the spinal cord (Evans, Francis, Jones, Smith & Watkins, 1982).

Currents up to 100 nA ejecting GDEE had inconsistent effects on excitations induced by the specific agonists. GDEE did not significantly antagonize any of the excitations of the cells reported in Table 1. However with seven other cells which were excluded from Table 1 because of incomplete recovery (< 80 % of pre-treatment firing rate), GDEE tended to antagonize responses to all three agonists, an effect which was sometimes paralleled by a decrease in spike height.

In the spinal cord and higher centres compounds which antagonize the NMA receptor tend also to antagonize L-aspartate-induced excitations, leaving L-glutamate relatively unaffected (Hicks, Hall & McLennan, 1978; Lodge, Headley & Curtis, 1978; Collingridge & Davies, 1979; McLennan & Lodge, 1979). Table 1 indicates that the

same trend occurs also in the hippocampus. A dose of either APV (Fig. 3*B*) or DGG which produced almost complete inhibition of the NMA response also markedly depressed the L-aspartate response but had little or no effect on the glutamate reaction. Interestingly with lower doses of APV or DGG (Fig. 5*A*) it was often possible effectively to antagonize the NMA-induced response without appreciably affecting the L-aspartate reaction (McLennan & Liu, 1982).

When DGG was applied at a dose which reduced both L-glutamate-and L-aspartate-induced excitations, the latter were more sensitive (Fig. 5*B*). This observation is consistent with the sensitivities of NMA and quisqualate receptors to DGG antagonism.

An effect qualitatively similar to that observed with APV and DGG was seen also with D-APB, that is to say the NMA and L-aspartate, but not the L-glutamate, responses were antagonized (Table 1, Fig. 4*B*). The L-(+)-isomer of APB had no significant effect on excitations induced by L-aspartate and L-glutamate (Table 1, Fig. 4*B*.

When applied with 100 nA ejecting currents DGG, APV and D-APB had no significant effect on the excitations induced by ACh (Table 1).

## DISCUSSION

The involvement of post-synaptic excitatory amino acid receptors in stratum radiatum, where the Schaffer collateral–commissural input to the CA1 pyramidal cells terminates (Laurberg, 1979), was implicated by the existence of foci at which ionophoresis of L-glutamate, L-aspartate and quisqualate elicited short latency firing (cf. Dudar, 1974; Schwartzkroin & Andersen, 1975; Spencer, Gribkoff & Lynch, 1978). Qualitatively different CA1 responses to NMA and kainate were observed at the same sites. The slow continuous rise of kainate-mediated firing could be due to the absence of low affinity glial or neuronal uptake systems (Johnston, Kennedy & Twitchin, 1979) such as exist for L-glutamate, L-aspartate (Balcar & Johnston, 1973) and possibly for quisqualate (Lodge, Curtis, Johnston & Bornstein, 1980). The presence of only a weak uptake mechanism for NMA (Skerritt & Johnston, 1981) similarly may explain its tendency to recruit adjacent neurones and eventually to produce high frequencies of firing but is not a reasonable explanation of its unique ability to induce the bursting pattern of firing of CA1 neurones. A similar bursting response was not observed by us in dentate granule cells (unpublished observation) and has not been reported by other investigators as occurring with NMA elsewhere in the C.N.S.

As in other regions, APV was found to be a potent and selective antagonist of the NMA-induced excitation of hippocampal CA1 neurones; but typically there was a concomitant potentiating effect on the excitations induced by kainate and quisqualate. Both of these effects appeared to be exerted by the D-(−)-isomer. The effects of DAA were qualitatively the same as those observed with its $\omega$-phosphonate analogue APV. In other C.N.S. regions DAA has been reported to enhance the effect of quisqualate (Evans, Francis, Hunt, Oakes & Watkins, 1979) and glutamate (Lodge *et al.* 1978); to increase spontaneous activity of the dentate gyrus (Hicks & McLennan, 1979) and spinal cord (Lodge *et al.* 1978); and to depolarize frog spinal neurones (Evans, Francis & Watkins, 1978). These observations imply that DAA has subliminal

excitatory effects, and similarly the enhancement by APV of quisqualate and kainate effects is suggestive of a weak excitatory action. However, APV did not potentiate responses to ACh, and no direct evidence for an excitatory effect of APV has been obtained.

That low ionophoretic doses of the dipeptide DGG antagonized responses to kainate (as well as NMA and L-aspartate) but not quisqualate, supports the proposed interaction of kainate and quisqualate with two different receptors (Davies & Watkins, 1981). However, there was a significant reduction of the action of quisqualate when higher ejecting currents of DGG were used, indicating that this substance may be useful as a quisqualate antagonist, especially since other potential quisqualate antagonists (GDEE and PDA) appear not useful in the hippocampus. The decline of the L-glutamate response concomitant with the antagonism of quisqualate by DGG is consistent with a preferential reaction of L-glutamate with quisqualate receptors (McLennan, Hicks & Liu, 1982).

Previous reports of APB inhibition of synaptic responses in the hippocampus (Dunwiddie, Madison & Lynch, 1978; White, Nadler & Cotman, 1979; Koerner & Cotman, 1981) have assumed antagonism of amino acid receptors to be the underlying cause. Our results indicate that quite different effects are produced by the two isomers of APB. In agreement with the results of Evans *et al.* (1979) obtained with DL-APB in the frog spinal cord and those of Hori, Auker, Braitman & Carpenter (1981) in the rat olfactory cortex, D-APB antagonized NMA and kainate to a similar extent. More recent spinal cord studies (Davies & Watkins, 1982) demonstrated non-specific antagonism by D-APB of amino acid-induced responses which may simply reflect dose differences. As with APV and DGG, D-APB antagonism of the NMA response paralleled a decline, albeit smaller, in the L-aspartate response (but see Hori *et al.* 1981). Typically the effect of L-APB was to enhance amino acid-induced responses and occasionally to excite cells by itself. Blockade of the agonist effects of L-APB by D-APB in this study and by D-APB and APV in the isolated spinal cord preparation (Evans *et al.* 1982) imply that L-APB excitation is mediated through the NMA receptor.

Quite clearly inhibition of synaptic transmission by L-APB in the hippocampal formation cannot be attributed to antagonism of any one of the three 'conventional' amino acid receptors. Our observation that L-APB did not antagonize L-glutamate-induced excitations also argues against its interaction with a novel glutamate receptor but does not preclude antagonism of the receptor for an unidentified neurotransmitter.

Although it was a potent, non-selective amino acid antagonist PDA also had a direct APV-sensitive excitatory effect on CA1 neurones (cf. Segal, 1981). Partial NMA-like PDA agonist effects have been observed in the spinal cord (Davies *et al.* 1981*a*). Interpretation of actions on synaptic mechanisms would be complicated by this depolarizing action.

Although GDEE has a demonstrated ability to antagonize quisqualate responses selectively in the spinal cord *in vivo* (Davies & Watkins, 1979; McLennan & Lodge, 1979; but see Evans *et al.* 1979) the results of this study support the conclusion of White *et al.* (1979) who described its effects as 'capricious and inexplicable'. This is in contrast to the reports of Spencer *et al.* (1976) for CA1 neurones and of Hicks & McLennan (1979) and Wheal & Miller (1980) for the dentate gyrus where reasonable

specificities were found. Whatever the explanation for these discrepancies may be, because its actions are unclear the value of GDEE as an antagonist of excitatory synaptic input to CA1 seems limited.

In conclusion the effects of the antagonists on amino acid-induced excitations of CA1 neurones in general conform to those reported for the spinal cord. The data show that of the antagonists tested DGG and APV may be useful in synaptic investigations while others such as D- and L-APB, PDA and GDEE may be of lesser value because they are less potent and/or have complicating direct effects on CA1 cells.

## REFERENCES


BALCAR, V. J. & JOHNSTON, G. A. R. (1973). High affinity uptake of transmitters: Studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in cat spinal cord. *J. Neurochem.* **20**, 529–539.

COLLINGRIDGE, G. L. & DAVIES, J. (1979). An evaluation of D-α-aminoadipate and D- (and DL) α-aminosuberate as selective antagonists of excitatory amino acids in the substantia nigra and mesencephalic reticular formation of the rat. *Neuropharmacology* **18**, 193–199.

COLLINGRIDGE, G. L., KEHL, S. J. & MCLENNAN, H. (1982). Antagonism of amino acid-induced excitation of CA1 hippocampal neurones *in vitro*. *J. Physiol.* **322**, 52*P*.

COLLINGRIDGE, G. L., KEHL, S. J. & MCLENNAN, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus. *J. Physiol.* **334**, 33–46.

DAVIES, J., EVANS, R. H., FRANCIS, A. A., JONES, A. W. & WATKINS, J. C. (1981*a*). Antagonism of excitatory amino acid-induced and synaptic excitation of spinal neurones by *cis*-2,3-piperidine dicarboxylate. *J. Neurochem.* **36**, 1305–1307.

DAVIES, J., FRANCIS, A. A., JONES, A. W. & WATKINS, J. C. (1981*b*). 2-amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neurosci. Lett.* **21**, 77–81.

DAVIES, J. & WATKINS, J. C. (1979). Selective antagonism of amino acid induced and synaptic excitation in the cat spinal cord. *J. Physiol.* **297**, 621–635.

DAVIES, J. & WATKINS, J. C. (1981). Differentiation of kainate and quisqualate receptors in cat spinal cord by selective antagonism with γ-D (and L)-glutamylglycine. *Brain Res.* **206**, 172–177.

DAVIES, J. & WATKINS, J. C. (1982). Actions of D and L forms of 2-amino-5-phosphonovalerate and 2-amino-4-phosphonobutyrate in the cat spinal cord. *Brain Res.* **235**, 378–386.

DINGLEDINE, R., DODD, J. & KELLY, J. S. (1980). The *in vitro* brain slice as a useful neurophysiological preparation for intracellular recording. *J. Neurosci. Meth.* **2**, 323–362.

DODD, J., DINGLEDINE, R. & KELLY, J. S. (1981). The excitatory action of acetylcholine on hippocampal neurones of the guinea pig and rat maintained *in vitro*. *Brain Res.* **207**, 109–127.

DUDAR, J. D. (1974). *In vitro* excitation of hippocampal pyramidal cell dendrites by glutamic acid. *Neuropharmacology* **13**, 1083–1089.

DUNWIDDIE, T., MADISON, D. & LYNCH, G. (1978). Synaptic transmission is required for initiation of long-term potentiation. *Brain Res.* **150**, 413–417.

EVANS, R. H., FRANCIS, A. A., HUNT, K., OAKES, D. J. & WATKINS, J. C. (1979). Antagonism of excitatory amino acid-induced responses and of synaptic excitation in the isolated spinal cord of the frog. *Br. J. Pharmac.* **67**, 591–603.

EVANS, R. H., FRANCIS, A. A., JONES, A. W., SMITH, D. A. S. & WATKINS, J. C. (1982). The effects of a series of ω-phosphonic α-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. *Br. J. Pharmac.* **75**, 65–76.

EVANS, R. H., FRANCIS, A. A. & WATKINS, J. C. (1978). $Mg^{2+}$-like selective antagonism of excitatory amino acid-induced responses by α,ε-diaminopimelic acid, D-α-aminoadipate and HA-966 in isolated spinal cord of frog and immature rat. *Brain Res.* **148**, 536–542.

HICKS, T. P., HALL, J. G. & MCLENNAN, H. (1978). Ranking of excitatory amino acids by the antagonists glutamic acid diethylester and D-α-aminoadipic acid. *Can. J. Physiol. Pharmac.* **56**, 901–907.

HICKS, T. P. & MCLENNAN, H. (1979). Amino acids and the synaptic pharmacology of granule cells in the dentate gyrus of the rat. *Can. J. Physiol. Pharmac.* **57**, 973–978.

HONORÉ, T., KROGSGAARD-LARSEN, P., HANSEN, J. J. & LAURIDSEN, J. (1981). Glutamate and aspartate agonists structurally related to ibotenic acid. *Molec. cell. Biochem.* **38**, 123–128.

HORI, N., AUKER, C. R., BRAITMAN, D. J. & CARPENTER, D. O. (1981). Lateral olfactory tract transmitter: Glutamate, aspartate or neither? *Cell molec. Neurobiol.* **1**, 115–120.

JOHNSTON, G. A. R., KENNEDY, S. M. E. & TWITCHIN, B. (1979). Action of the neurotoxin kainic acid on high affinity uptake of L-glutamic acid in rat brain slices. *J. Neurochem.* **32**, 121–127.

KOERNER, J. F. & COTMAN, C. W. (1981). Micromolar L-2-amino-4-phosphonobutyric acid selectively inhibits perforant path synapses from lateral entorhinal cortex. *Brain Res.* **216**, 192–198.

LAURBERG, S. (1979). Commissural and intrinsic connections of the rat hippocampus. *J. comp. Neurol.* **184**, 685–708.

LODGE, D., CURTIS, D. R., JOHNSTON, G. A. R. & BORNSTEIN, J. C. (1980). *In vivo* inactivation of quisqualate: studies in the cat spinal cord. *Brain Res.* **182**, 491–495.

LODGE, D., HEADLEY, P. M. & CURTIS, D. R. (1978). Selective antagonism by D-$\alpha$-aminoadipate of amino acid and synaptic excitation of cat spinal neurons. *Brain Res.* **152**, 603–608.

LYNCH, G. & SCHUBERT, P. (1980). The use of *in vitro* brain slices for multidisciplinary studies of synaptic function. *A. Rev. Neurosci.* **3**, 1–22.

MCLENNAN, H. (1981). On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system. In *Glutamate as a Neurotransmitter*, ed. DI CHIARA, G. & GESSA, G. L., pp. 253–262. New York: Raven Press.

MCLENNAN, H. (1982) The isomers of 2-amino-5-phosphonovalerate as excitatory amino acid antagonists – a reappraisal. *Eur. J. Pharmac.* **79**, 135–137.

MCLENNAN, H., HICKS, T. P. & LIU, J. R. (1982). On the configuration of the receptors for excitatory amino acids. *Neuropharmacology* **21**, 549–544.

MCLENNAN, H. & LIU, J. R. (1982). The action of six antagonists of the excitatory amino acids on neurones of the rat spinal cord. *Exp. Brain Res.* **45**, 151–156.

MCLENNAN, H. & LODGE, D. (1979). The antagonism of amino acid-induced excitation of spinal neurones in the cat. *Brain Res.* **169**, 83–90.

NADLER, J. V., VACA, K. W., WHITE, W. F., LYNCH, G. S. & COTMAN, C. W. (1976). Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. *Nature, Lond.* **270**, 743–745.

SCHWARTZKROIN, P. A. (1975). Characteristics of CA1 neurons recorded intracellularly in the hippocampal *in vitro* slice preparation. *Brain Res.* **85**, 423–435.

SCHWARTZKROIN, P. A. & ANDERSEN, P. (1975). Glutamic acid sensitivity of dendrites in hippocampal slices *in vitro*. *Adv. Neurol.* **12**, 45–51.

SEGAL, M. (1981). The actions of glutamic acid on neurons in the rat hippocampal slice. In *Glutamate as a Neurotransmitter*, ed. DI CHIARA, G. & GESSA, G. L., pp. 217–225. New York: Raven Press.

SKERRITT, J. H. & JOHNSTON, G. A. R. (1981). Uptake and release of *N*-methyl-D-aspartate by rat brain slices. *J. Neurochem.* **36**, 881–885.

SPENCER, H. J., GRIBKOFF, V. K., COTMAN, C. W. & LYNCH, G. S. (1976). GDEE antagonism of iontophoretic amino acid excitations in the intact hippocampus and in the hippocampal slice preparation. *Brain Res.* **105**, 471–481.

SPENCER, H., GRIBKOFF, V. K. & LYNCH, G. S. (1978). Distribution of acetylcholine, glutamate and aspartate sensitivity over the dendritic fields of hippocampal CA1 neurones. In *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System*, ed. RYALL, R. W. & KELLY, J. S., pp. 194–196. Amsterdam: Elsevier/North Holland Biomedical Press.

STORM-MATHISEN, J. (1977). Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog. Neurobiol.* **8**, 119–181.

STORM-MATHISEN, J. (1981). Glutamate in hippocampal pathways. In *Glutamate as a Neurotransmitter*, ed. DI CHIARA, G. & GESSA, G. L., pp. 43–55. New York: Raven Press.

WATKINS, J. C. (1981*a*). Pharmacology of excitatory amino acid receptors. In *Glutamate: Transmitter in the Central Nervous System*, ed. ROBERTS, P. J., STORM-MATHISEN, J. & JOHNSTON, G. A. R., pp. 1–24. New York: John Wiley and Sons.

WATKINS, J. C. (1981*b*). Pharmacology of excitatory amino acid transmitters. In *Amino Acid Neurotransmitters*, ed. DE FEUDIS, F. V. & MANDEL, P., pp. 205–212. New York: Raven Press.

WHEAL, H. V. & MILLER, J. J. (1980). Pharmacological identification of acetylcholine and glutamate excitatory systems in the dentate gyrus of the rat. *Brain Res.* **182**, 145–155.

WHITE, W. F., NADLER, J. V. & COTMAN, C. W. (1979). The effect of acidic amino acid antagonists on synaptic transmission in the hippocampal formation *in vitro*. *Brain Res.* **164**, 177–194.